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13. ABSTRACT *(Maximum 200 Words)*

We hypothesized that aberrant expression of Hox genes in mammary epithelial cells results in the inappropriate regulation of a wide variety of Hox target genes; this could lead to a change from a normal to a preneoplastic, or from a preneoplastic to a neoplastic phenotype. The purpose is to test this hypothesis by manipulating the expression of Hox genes such as Hoxa1, Hoxa3, or Hoxa11 in cells from hyperplastic (preneoplastic) lesions of the breast by employing a tetracycline regulated vector system. The cells will be assayed for growth, response to retinoic acid (RA), and their expression of markers of the neoplastically transformed state. The goals of this research are: (1) to understand the functions of homeobox genes in normal and neoplastically transformed mammary epithelial cells; (2) to ascertain whether or not the overexpression of homeobox genes results in a neoplastic phenotype; and (3) to assess whether these Hox gene overexpressing cells respond to exogenous RA. In the past year progress has been made, including analysis of various murine tumor samples for Hox gene expression. The significance is that this new information may be employed to develop novel methods for regulating homeobox gene expression as a therapy for human breast cancer.

14. SUBJECT TERMS
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4) INTRODUCTION

This hypothesis is built upon the observations that Hox genes are broadly-active transcriptional regulators in tissue and organ developmental processes, that some of these genes are specifically upregulated in neoplastic cells and not in preneoplastic cells, that neoplastic cells show massive deregulation of gene expression, and that neoplastic cells interact with their local environment much like cells in organogenesis. The hypothesis states that aberrant expression of Hox genes, such as *Hoxa11*, in mammary epithelial cells results in major deregulation of gene expression and is a significant contributing factor to the neoplastic phenotype. The goals of this proposed research are to gain new insights into the functions of specific Hox genes with respect to the regulation of the growth of mammary epithelial cells using cell culture and animal model systems. These experiments are underway. The purpose of the research is to gain new knowledge about the intracellular molecular events which occur in the process of mammary carcinogenesis and to utilize this knowledge develop new therapies for breast cancer. The scope of this research includes two laboratories with complementary expertise, and both cell culture and mouse models of breast carcinogenesis. This annual report is being written in month 11 of this project.

5) BODY

Task 1. To examine the consequences of Hox gene overexpression on the phenotype of mammary epithelial cells in cell culture (months 1-14).

- Cloning of Hox genes into pUHD-10-3 (months 1-3)

- Generation and initial characterization of cell lines which stably express both pUHD-10-3/"Hox gene of interest" (i.e. Hoxa3 or Hoxa11, for example) and the tTA protein under tetracycline control (months 3-8)

- Assay of cell growth rate, cell morphology, cloning efficiency, and response to retinoic acid (months 9-14)

In Task 1, we are artificially manipulating the expression of specific Hox genes in cells from hyperplastic (pre-neoplastically transformed) lesions by employing the tetracycline-inducible vector system (1-3). Full-length murine cDNA clones for the Hoxa1, Hoxa3, and Hoxa11 genes are available in this laboratory and are being inserted into the plasmid pUHD-10-3, which contains a heptamerized tet operator upstream of a CMV minimal promoter; this is followed by a multiple cloning site and an SV40 polyadenylation sequence 3' of the multiple cloning site. The TM10 cell line is being used as the initial transfection recipients; our first priority is to analyze Hoxa1 and Hoxa11 (4). TM10 cells are diploid, have a polygonal morphology in culture, and grow in a cobblestone pattern typical of mammary epithelial cells. The TM10 preneoplastic outgrowth from which this cell line was derived has modest tumorigenic potential and is classified as a stage II hyperplasia. Cultured TM10 cells are stably transfected by electroporation with this construct in addition to the tetracycline expression vector PTA-N, which contains the tetracycline/VP16 transactivator gene under the control of a tet heptamer sequence (1-3). This allows both the Hox protein of interest and the tet-VP16 transactivator protein to be regulated by the drug tetracycline. In the presence of tetracycline, no transactivator message or Hox cDNA should be expressed, but when tetracycline is removed from the medium the Hox gene expression should be greatly induced. Stable cell lines which have been shown by Northern blot to exhibit inducible expression of the Hox gene are then analyzed in culture with respect to their cell morphology, growth rate ([3H]thymidine uptake, cell number) over a 7-day period, and colony formation efficiency (14-day assay) (5). In the cell culture system, we will also determine whether the hyperplastic cells are sensitive to growth inhibition by retinoic acid when they are overexpressing the homeobox gene of interest driven by the tetracycline promoter system (i.e. the removal of tetracycline will induce the

expression of the homeobox gene of interest); cell growth experiments will be performed with stably transfected, hyperplastic cell strains cultured ± retinoic acid, ± tetracycline. Cells stably transfected with only the PTA-N vector will be used as a control.

This research is in progress, as expected for months 1-11, according to the timetable proposed in the original application for Task 1 (months 1-14). The TM10 cells have been cultured and transfected with Hoxa1.

We have also examined homeobox gene expression in mammary cell lines from a related animal model of mammary carcinogenesis, the p53 -/- mouse. Altered expression of the p53 tumor suppressor gene is observed often in human breast cancers, but mammary tumors were seldom observed in p53 -/- mice (6). Dr. Medina and Dr. Gudas's laboratories have analyzed Hox gene expression in cells derived from p53 -/- mammary epithelium transplanted into the clear mammary fat pads of wild type p53 BALB/c hosts. The mice were then left untreated, stimulated with hormones from pituitary isografts, or treated with the carcinogen DMBA (7). The cultured cells from this model expressed several different Hox mRNAs at higher levels than those from control, wild type mice. We do not have enough data from enough different cell lines to apply statistical tests at this time.

We are progressing with this research at the rate projected in the original proposal. There have been no negative findings nor any significant problems in accomplishing this task.

Task 2. To examine the consequences of Hox gene expression on the growth of epithelial cells in an animal model; tumor incidence ± tetracycline will be assessed (months 9-36).

As we are now in month 11, we are just beginning this task.

Task 3. To examine gene expression in the Hox overexpressing cells. The tumor samples will be assayed directly from the animal, and cell lines will be made from the mammary carcinomas which overexpress different Hox genes, i.e. the tumors generated in Task 2. Gene expression studies aimed at measuring markers such as gelsolin, PKCδ, p96, C/EBPβ3, and cyclin D1 will be examined (months 18-36).

This task has not been initiated at this time (month 11).

6) KEY RESEARCH ACCOMPLISHMENTS

(a) Measurement and detection of homeobox (Hox) gene expression, including Hoxa1, Hoxa3, and Hoxa5, at the mRNA level in various murine mammary cell lines at various stages in the carcinogenesis process in wild type (WT) mice.

(b) Detection of Hox gene expression at the mRNA level in cultured mammary cell lines from p53 -/- mammary epithelium in the mammary fat pads from WT mice.

(c) Cloning of the Hoxa1 full-length cDNA into pUHD-10-3.

(d) Transfection of TM10 cells with the Hoxa1 gene in the vector generated in (c).

7) REPORTABLE OUTCOMES

Chen, S.W., Medina, D., and Gudas, L.J. Homeobox Gene Expression in Cultured Murine Mammary Cells from Various Stages in the Process of Carcinogenesis, in preparation.

Several stably transfected cell lines, derived from TM10, are being generated.

8) CONCLUSIONS

We have demonstrated in two different mammary tumorigenesis animal models that homeobox gene expression is aberrantly high relative to expression in normal, control, non-tumorigenic and/or non-hyperplastic mammary cells. These data are important for understanding why breast epithelial cells sometimes become deranged and form tumors. In the p53 -/- model, we also have found effects of hormones on the expression of Hox genes. Another laboratory has also reported related data (8), though they showed that Hoxa5 was required for p53 expression. In terms of the "so what section," these data are so far related to altered gene expression in tumor vs. normal cells. In addition to the gain of fundamental knowledge about the process of carcinogenesis, these Hox genes could possibly be biomarkers of different stages of the carcinogenesis process and/or targets for future pharmacological or gene therapy for breast cancer treatment.

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